Monsanto

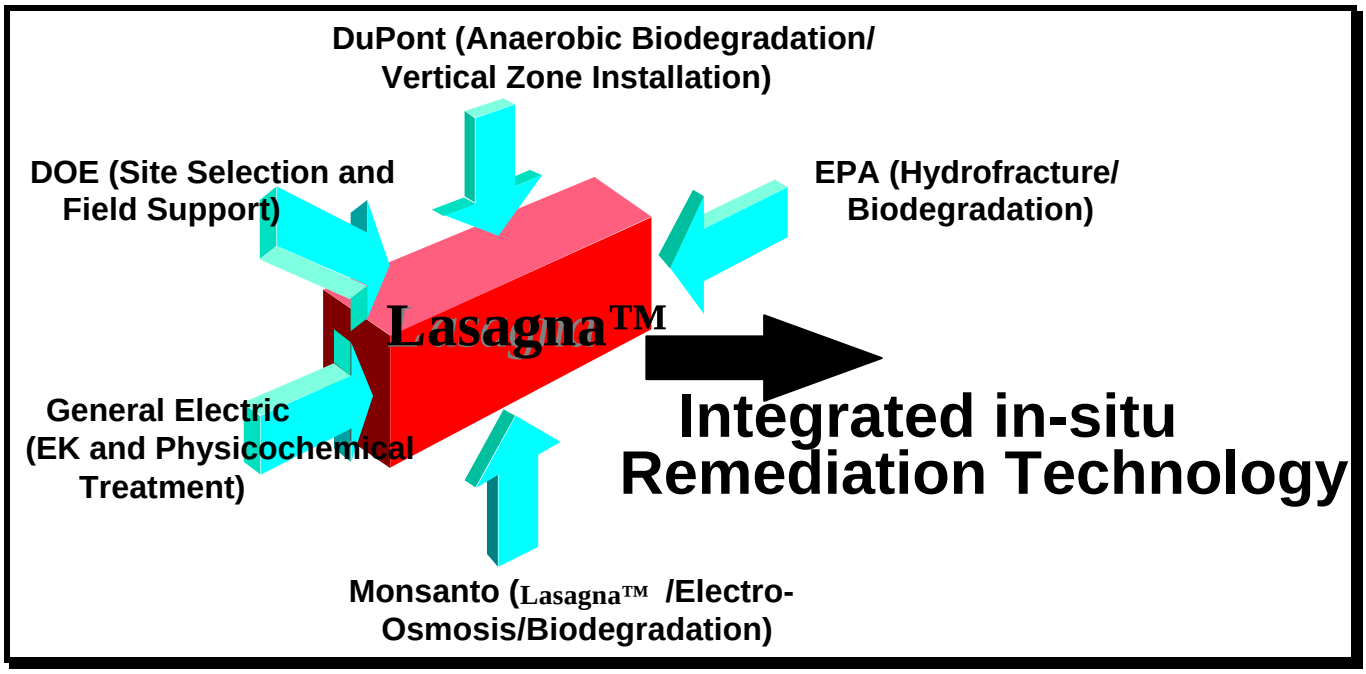
June 1996

Development of an Integrated *in-situ* Remediation Technology

Topical Report for *Task No. 6 entitled "Lab-Scale Development of Microbial Degradation Process" (September 26, 1994 - May 25, 1996)*

J. Martin Odom (DuPont Company)

DOE Contract Number: DE-AR21-94MC31185



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20 March, 1997

Re: Ordering Information for *"Development of an Integrated in-situ Remediation Technology"* Topical Reports generated under DOE contract number DE-AR21-94MC31185 which was signed September 26, 1994.

Dear Sir/Ms.:

The following table summarizes ordering information for all technical reports written for the above referenced contract. Copies of these reports may be obtained from the Office of Scientific and Technical Information [(423)576-8401] if you are with DOE or a DOE contractor, or from:

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(continued on next page)

Title	Document Number
• **Topical Report for Task #1 entitled "Evaluation of Treatment Zone Formation Options" (September 26, 1994 - May 25, 1996)** Stephen H. Shoemaker, Richard C. Landis, Ronald J. Griffith, Dale S. Schultz, and Gary E. Quinton (DuPont Company)	**DOE/METC/31185 —5436, DE97002165**
• **Topical Report for Tasks #2-4 entitled "Electrokinetic Modeling" (September 26, 1994 - May 25, 1996)** Andrew P. Shapiro (General Electric Company)	**DOE/METC/31185 —5391, DE97002135**
• **Topical Report for Task #5 entitled "Cost Analysis" (September 26, 1994 - May 25, 1996)** Gary Quinton, Dale Schultz, Richard Landis, Ronald Griffith, and Stephen Shoemaker (DuPont Company)	**DOE/METC/31185 —5389, DE97002134**
• **Topical Report for Task #6 entitled "Lab-Scale Development of Microbial Degradation Process" (September 26, 1994 - May 25, 1996)** J. Martin Odom (DuPont Company)	**DOE/METC/31185 —5388, DE97002130**
• **Topical Report for Task #7 entitled "Development of Degradation Processes" (September 26, 1994 - May 25, 1996)** M. J. Brackin, M. H. Heitkamp and S. V. Ho (Monsanto Company)	**DOE/METC/31185 —5495, DE97002165**
• **Topical Report for Tasks #8 and 10 entitled "Laboratory and Pilot Scale Experiments of the *Lasagna*™ Process" (September 26, 1994 - May 25, 1996)** Sa V. Ho, Christopher J. Athmer, and P. Wayne Sheridan (Monsanto Company) and Andrew P. Shapiro (General Electric Company)	**DOE/METC/31185 —5375, DE97002150**
• **Topical Report for Task #9-Part I entitled "TCE Degradation Using Non-Biological Methods" (September 26, 1994 - May 25, 1996)** Andrew P. Shapiro, Timothy M. Sivavec, and Sunita S. Baghel (General Electric Company)	**DOE/METC/31185 —5392, DE97002133**
• **Topical Report for Task #9 - Part II entitled "TCE Degradation Using Non-Biological Methods" (September 26, 1994 - May 25, 1996)** Robert G. Orth and David E. McKenzie (Monsanto Company)	**DOE/METC/31185 —5393, DE97002131**

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• **Topical Report for Task #11 entitled "Evaluation of TCE Contamination Before and After the Field Experiment" (September 26, 1994 - May 25, 1996)** B. Mason Hughes, Sa V. Ho, Christopher J. Athmer, and P. Wayne Sheridan (Monsanto Company) Stephen H. Shoemaker and John R. Larson (DuPont) Jay L. Clausen (LMES) and John L. Zutman (ORNL-Grand Junction)	**DOE/METC/31185—5496, DE97002166**
• **Topical Report for Tasks #12 and 13 entitled "Large Scale Field Test of the *Lasagna*™ Process" (September 26, 1994 - May 25, 1996)** Christopher J. Athmer, Sa V. Ho, B. Mason Hughes, P. Wayne Sheridan, and P. H. Brodsky (Monsanto Company) Andrew P. Shapiro, Roy F. Thornton, and Joseph J. Salvo (General Electric Company) and Dale S. Schultz, Richard C. Landis, Ron Griffith, and Stephen H. Shoemaker (DuPont)	**DOE/METC/31185—5390, DE97002156**

A. Executive Summary

Development of an Integrated *In Situ* Remediation Technology

DOE Contract Number: DE-AR21-94MC31185

Topical Report for *Task #6: "Lab-Scale Development of Microbial Degradation Process"*

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Abstract: Contamination in low permeability soils poses a significant technical challenge to in situ remediation efforts. Poor accessibility to the contaminants and difficulty in delivery of treatment reagents have rendered existing in situ treatments such as bioremediation, vapor extraction, and pump and treat rather ineffective when applied to low permeability soils present at many contaminated sites. The technology is an integrated in situ treatment in which established geotechnical methods are used to install degradation zones directly in the contaminated soil, and electro-osmosis is utilized to move the contaminants back and forth through those zones until the treatment is completed. The present Topical Report for Task #6 summarizes the results of a study of the potential for stimulating microbial reductive dehalogenation as part of the integrated in situ treatment process at the field experiment test site at DOE's Gaseous Diffusion Plant in Paducah, Kentucky. A series of "microcosm bottle tests" were performed on samples of contaminated soil and groundwater taken from the Paducah site and spiked with trichloroethene (TCE). A number of bottles were set up, each spiked with a different carbon source in order to enhance the growth of different microbial subpopulations already present within the indigenous population in the soil. In addition, a series of bottle tests were completed with samples of the granular activated carbon (GAC) treatment zone material retrieved from the test site during the Paducah field experiment. In these tests, the GAC samples were used in place of the soil. Results of the soil-groundwater microcosms yielded a negative indication of the presence of dechlorinating bacteria at the site. However, charcoal (GAC) samples from one location in the test plot exhibited marked dechlorination with conversion of TCE to dichloroethene.

B. Acronyms and Abbreviations

DOE	Department of Energy
GC/MS	gas chromatograph/mass spectrograph
GE	General Electric Company
DCE	dichloroethene
PCE	tetrachloroethene
TCE	trichloroethene
VOC	volatile organic compound
VC	vinyl chloride

C. Units

C	Centigrade
ft	feet
g	gram
hr	hour(s)
L	liter
m	meter
mg	milligram
mL	milliliter
mm	millimeter
mM	millimole
ppb	parts per billion
ppm	parts per million
µL	microliter

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E. Background

Statement of the Problem

Contamination in low permeability soils poses a significant technical challenge to *in-situ* remediation efforts. Poor accessibility to the contaminants and difficulty in delivery of treatment reagents have rendered existing *in-situ* treatments such as bioremediation, vapor extraction, and pump and treat, rather ineffective when applied to low permeability soils present at many contaminated sites.

The Solution

The proposed technology combines electro-osmosis with treatment zones that are installed directly in the contaminated soils to form an integrated *in-situ* remedial process. Electro-osmosis is an old civil engineering technique and is well known for its effectiveness in moving water uniformly through low-permeability soils with very low power consumption. Conceptually, the integrated technology could treat organic and inorganic contamination, as well as mixed wastes. Once developed, the technology will have tremendous benefits over existing ones in many aspects including environmental impacts, cost effectiveness, waste generation, treatment flexibility, and breadth of applications.

Consortium Description

A Consortium has been formed consisting of Monsanto, E. I. du Pont de Nemours & Co., Inc. (DuPont) and General Electric (GE), with participation from the Environmental Protection Agency (EPA) Office of Research and Development and the Department of Energy (DOE) Environmental Management Office of Science and Technology. The five members of this group are leaders in their represented technologies and hold significant patents and intellectual property which, in concert, may form an integrated solution for soil treatment. The Consortium's activities are being facilitated by Clean Sites, Inc., under a Cooperative Agreement with EPA's Technology Innovation Office. A schematic diagram of the government/industry consortium is shown on the front page of this topical report.

Management Plan

A Management Plan for this project was prepared by Monsanto and submitted on November 30, 1994. That plan summarized the work plan which was developed in conjunction with DuPont, GE, EPA's Risk Reduction Engineering Laboratory (RREL), Martin Marietta Energy Systems (MMES), and the Department of Energy. The DOE Gaseous Diffusion Plant in Paducah, Kentucky, has been chosen as the site for the initial field tests. CDM Federal Programs Corporation was chosen to provide the on-site support of the field tests which were installed at the DOE site in November 1994. This experiment tested the combination of electro-osmosis and *in-situ* sorption in the treatment zones. In 1994 and 1995, technology development was carried out under the present contract by Monsanto, DuPont, and GE. These studies evaluated various degradation processes and their integration into the overall treatment scheme at bench and pilot scales.

Technical Deliverables

Tables 1 and 2 summarize the 13 technical tasks and the 8 topical reports which will be written describing the results obtained in the technical tasks. These two tables show

which organization is primarily responsible for the tasks and for preparing the topical reports. The present topical report summarizes Task #6 - Lab-Scale Development of Microbial Degradation Process.

Table E-1. List of Tasks and Responsible Company

Task	Company
Task 1 - Evaluation of Treatment Zone Formation Options (5.1.2)	DuPont
Task 2 - Electrokinetic Model Validation and Improvement (6.5)	GE
Task 3 - Design Guidance for Field Experiments (6.6)	GE/DuPont
Task 4 - Analysis of Electrode Geometry and Soil Heterogeneity (6.7)	GE/DuPont
Task 5 - Cost Analysis (7)	Monsanto/DuPont
Task 6 - Lab-Scale Development of Microbial Degradation Process (8.1.2)	DuPont
Task 7 - Lab-Scale Electrokinetic and Microbial Degradation (8.1.6)	Monsanto
Task 8 - Lab-Scale Tests of Lasagna Process Using DOE Paducah Soil (8.1.7)	Monsanto
Task 9 - TCE Degradation Using Non-Biological Methods (8.2.1, 8.2.2.2, 8.2.3.2)	GE/Monsanto
Task 10 - Bench- and Pilot-Scale Tests (9.3)	Monsanto
Task 11 - Establish Contamination Conditions Before and After Tests (10.1.2)	DuPont/MMES
Task 12 - Design and Fabrication of Large-Scale Lasagna Process (12.1, 12.2)	Monsanto/DuPont/Nilex
Task 13 - Large-Scale Field Test of Lasagna Process (12.3, 12.4)	Monsanto/CDM

Table E-2. List of Topical Reports and Responsible Company

Topical Report	Company
Task 1 - Evaluation of Treatment Zone Formation Options	DuPont
Tasks 2 - 4 Electrokinetic Modeling	GE
Task 5 - Cost Analysis	Monsanto
Task 6 - Laboratory-Scale Microbial Degradation	DuPont
Tasks 7, 8, 10 - Bench- and Pilot-Scale Tests of Lasagna Process	Monsanto
Tasks 9 - TCE Degradation Using Non-Biological Methods	GE
Task 11 - Contamination Analysis, Before and After Treatment	Monsanto
Tasks 12 and 13 - Large-Scale Field Test of Lasagna Process	Monsanto

F. TOPICAL REPORT FOR TASK #6

1.0 INTRODUCTION

1.1 Technical Background

Dehalogenation of chlorinated solvents is known to be a naturally occurring, microbial process, which may occur aerobically or anaerobically. The aerobic process, in which the solvent mineralizes to carbon dioxide, is well understood biochemically. However, *in situ* implementation is often problematic due to requirements for oxygen and specialized co-substrates. The anaerobic reductive process has been documented in both the laboratory and the field but is poorly understood at the molecular level. For any particular chlorinated solvent, the result is a sequential release of one free chloride anion and the corresponding dehalogenated hydrocarbon. The sequence below shows the dehalogenation of tetrachloroethene (PCE) through intermediates trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC) to ethylene:

$$\text{PCE} \xrightarrow[+2e^-]{-\text{Cl}} \text{TCE} \xrightarrow[+2e^-]{-\text{Cl}} \text{DCE} \xrightarrow[+2e^-]{-\text{Cl}} \text{VC} \xrightarrow[+2e^-]{-\text{Cl}} \text{ETHYLENE}$$

The microbiology and biochemistry around this sequence is only currently being resolved; however, it is becoming evident that anaerobic dehalogenation falls into two broad metabolic types. The first is anaerobic dehalogenation by co-metabolism where the molar ratio of dechlorination to natural respiration is very low (i.e., 1:100 to 1:1000). The second type appears to be able to utilize solvent as a major repository of reducing equivalents and, in the process, gain energy for growth via anaerobic respiration. The co-metabolic process is inefficient and does not "enrich" the dehalogenating organism because the microorganism derives no benefit from the dehalogenation. The “anaerobic solvent respirers” should be self-enriching when solvents are the sole available electron acceptor. At present there is no certain way to specifically stimulate a dehalogenating microbial population by adding a specific substrate. Therefore, a variety of nutritional conditions are employed in assessing the presence of dehalogenating bacteria at a given site. The present study assesses nutritional conditions that stimulate the anaerobic, reductive process.

1.2 Anaerobic Dehalogenation in the Field

Available field data suggest that anaerobic dehalogenation varies greatly from site to site as an intrinsic or naturally occurring process. The occurrence of the cis isomer of dichloroethene in the ground is, however, quite common and considered diagnostic of the anaerobic reductive process because of the preferential formation of this isomer over the trans form. The relative contribution of the co-metabolic process vs the respiratory process is unknown for any particular site.

1.3 Paducah Lasagna™ Pilot

The Paducah test site soils are primarily contaminated with low to moderate levels of TCE in the range of 10 to 100 ppm. There are no prior laboratory data for anaerobic reductive dehalogenation at this site; however, low levels of DCE are present in the aquifer matrix. The field evidence for intrinsic reductive dehalogenation is not compelling. Refer to Topical Reports for Tasks 11 and 12/13 for further details concerning site conditions.

A pilot test was constructed at this site to assess the feasibility of using electro-osmosis to induce groundwater and the associated solvent to move through a grid of activated charcoal where the solvent would be captured. This arrangement does not rely on microbial activity to degrade the solvent but does not preclude microbial degradation in either the soil or the charcoal. Bacteria are known to colonize activated charcoal, and in the present configuration at Paducah, there is nothing to prevent soil bacteria from colonizing the charcoal. In fact, anomalously high levels of DCE were found in the charcoal traps removed from the test plot. If dechlorinating bacteria were to find the charcoal a favorable environment, then actual *in situ* degradation of the solvent might be achieved in addition to enhanced electro-osmotic removal. The experiments detailed in this report were directed toward determining whether microbial degradation may be naturally superimposed on the electro-osmotic process.

1.4 Objective and Scope

The objective of this work was to determine whether the bacteria necessary for anaerobic reductive dehalogenation are present in the soil or the charcoal. The work was meant to determine the inherent potential for dechlorination at this site; however, natural rate and the extent of dehalogenation as it may occur in the ground cannot be accurately inferred from this approach. This approach establishes only that dehalogenating bacteria are or are not present; it may, however, infer a best substrate or condition for stimulation of dehalogenation.

1.5 Approach

The work described here is typically referred to as the "Microcosm Bottle Test." The test consists of filling glass bottles with sterile groundwater and then adding the soil to be tested; the soil, not the groundwater, is the source of microorganisms. A number of bottles were set up, each spiked with a different carbon source to generate a different microbial population; each bottle was spiked with additional solvent (TCE) before being sealed. The bottles were then sampled intermittently over time, and the samples were analyzed for volatile organic chlorinated compounds. The nutritional conditions in any single microcosm bottle may be designed to promote a unique microbial community; however, there will be no tendency to enrich or select for a specific microorganism in this format. The various nutritional amendments for the soil tests and charcoal tests are shown in Table F-1.

Table F-1. Nutritional Amendments		
	Soil Tests	**Charcoal Tests**
Yeast extract	Yes	Yes
Sucrose	Yes	No
Methanol	Yes	No
Benzoate	Yes	Yes
Acetate	Yes	Yes
No addition	Yes	Yes
Killed control	Yes	Yes

It was anticipated that substrates such as yeast extract and sucrose would result in very different microbial populations than acetate or methanol. Benzoate may be considered somewhat intermediate between yeast extract and acetate.

Controls consisted of unsterilized, unamended bottles as well as sterilized bottles.

2.0 METHODOLOGY

2.1 Soil Experiments

A native-clay soil sample was obtained from a hand-augured soil boring completed roughly 30 ft to the south of the Phase I Lasagna™ test site at Paducah. Soils were shipped in glass Bell jars that were completely filled with associated groundwater collected from a nearby shallow monitor well. Both soil and groundwater samples were obtained from the TCE-contaminated region and are considered representative of the contaminated soils addressed in the Phase I pilot study. Samples were shipped overnight at ambient temperatures.

Microcosms for soil experiments were set up as follows: 25 mL of sediment was combined with sufficient sterile groundwater to completely fill a 250 mL Wheaton bottle. The groundwater was filter-sterilized and made 2 mM in ammonium chloride, 2 mM potassium phosphate, and 0.002% Resazurin (Sigma Chemical, St. Louis) in all microcosm experiments. (Resazurin is a redox-sensitive dye, which is added to media to give a simple, qualitative indicator of the redox conditions of the media. The dye is pink when oxygen is present and colorless when the media is highly reduced. This color difference lets the experimenter know that the media is safe for strict anaerobes.) Filter-sterilized groundwater was used to preclude surface contamination. This contamination can grow significantly between the time the groundwater is drawn and the time it arrives in the laboratory. It was assumed that the bulk of the bacteria were adhering to the

sediments and not free in the water phase. Sterilized soil was obtained by autoclaving soil for 1 hr on three consecutive days. Final concentrations of organic amendments were:

- Yeast extract (0.05%).
- Methanol (24 mM).
- Sucrose (2 mM).
- Sodium benzoate (4 mM).
- Sodium acetate (12 mM).

Bottles with additional sulfate contained 20 mM sodium sulfate. Each microcosm was set up in duplicate. Bottles were spiked to 20 ppm TCE (3.1 µL) and then capped with Teflon®-lined stoppers (with crimp-seal) and allowed to stand for 48 hrs before the initial or zero-time sample was taken. The bottles were incubated in the dark at ambient or room temperature. Samples were taken by removing the stopper within an anaerobic glove bag and then removing 2 mL of liquid for VOC, methane, and sulfide assay. A 1-mL sample was combined with 0.5 mL of methanol and stored at -80 _C before GC/MS analysis. A 0.5-mL sample was used for methane and sulfide analysis each.

2.2 Charcoal Experiments

Charcoal samples for these experiments were obtained from different sections of the electro-osmotic cell (see Figure F-1). All samples were obtained from a depth of 12 ft and transported from the site to the lab in glass jars completely filled with associated groundwater. All samples consisted solely of activated charcoal without any of the material comprising the insert itself. Within the anaerobic chamber, a subsample of charcoal (5 g) from each jar was ground in a mortar and then mixed with 30 mL of the microcosm medium. The heavy particles in this mixture were allowed to settle, and the supernatant (28 mL) from this step was used as inoculum for the 250 mL nutritionally amended bottles. This step removed the charcoal, which can interfere with analytical methods, but released the bacteria trapped with the charcoal.

Sample designations were as follows:

- C-12, a control area outside of the electro-osmotic cell.
- C1-7-12, a charcoal cell located near the anode.
- C2-6-12, a charcoal cell located near the middle of the pilot area.
- C4-3-12, a charcoal cell located near the cathode.

Two types of nutritional amendments were tested with the following final concentrations:

- 0.05% yeast extract only.
- Mixture of 5 mM sodium acetate and 10 mM sodium benzoate.

Each microcosm was set up in duplicate. Bottles were spiked to 20 ppm TCE (3.1 µL) and then capped with Teflon®-lined stoppers (with crimp-seal) and allowed to stand for 48 hrs before the initial or zero-time sample was taken. The bottles were incubated in the dark at ambient or room temperature. Samples were taken by removing the stopper within an anaerobic glove bag and then removing 1.0 mL of sample, which was combined with 0.5 mL of methanol and stored at -80 _C before GC/MS analysis.

Figure F-1

3.0 RESULTS AND DISCUSSION

3.1 Soil Analyses

The addition of sulfate fundamentally changes the composition of the microbial community. Sulfate-amended cultures will have high populations of sulfate-reducing bacteria, but no methanogenic bacteria. Unamended cultures will have high populations of methanogens. The experiments were not designed to "enrich" for any organism, and thus it is unlikely that solvent respirers would have been a dominant type in the bottle tests unless they were already present in high numbers. The addition of sulfate certainly provides ample environment for co-metabolism of solvent with sulfate respiration. In short, the design of the experiment did not specifically inhibit the solvent respirers, but it was biased in favor of co-metabolic metabolism.

Figures F-2 through F-7 show the TCE levels, in ppm, for the soil microcosm. The time course for these experiments was 80 days. Initially, TCE concentrations were generally higher than anticipated, averaging closer to 30 ppm rather than the expected 20 ppm. The reasons for this are unclear; however, levels may have been higher than anticipated in the samples themselves. Three out of four unamended (two for without sulfate and two for with sulfate set) controls were reasonably stable (Figure F-2) during the experiment; only bottle 11 showed marked loss after 17 days. The killed controls displayed lower levels of TCE even though they initially received the same amount of TCE. The lower initial value could have been due to loss of indigenous TCE in the soil sample itself (after repeated autoclaving); however, again the overall retention of TCE in these samples was quite good over the time course of the experiment.

Figures F-3 through F-7 show the TCE levels in yeast extract (with/without sulfate); sucrose (with/without sulfate), benzoate (with/without sulfate), methanol (with/without sulfate), and acetate (with/without sulfate) microcosms. Yeast extract, sucrose, methanol, and benzoate microcosms showed small losses over time that were not correlated with sulfate or the organic amendment. The DCE or VC concentrations did not change in any of these microcosms. The acetate-amended microcosms all showed a significant decrease in TCE near day 51; there were no increases in DCE or VC at this time. Therefore, it is difficult to infer dechlorination in any microcosm bottle from these data.

3.2 Charcoal Analyses

Figure F-1 shows a schematic of the electro-osmosis test plot at Paducah and the corresponding points where samples were obtained. Figures F-8 through F-11 show the responses of the various samples to nutritional stimulation with either yeast extract or benzoate plus acetate. These were compromise amendments designed to span the range of microbial types with a minimal number of microcosm bottles. No additional sulfate concentrations were tested in these experiments since no apparent beneficial or stimulatory effect, due to sulfate, was observed in the soil tests.

Figures F-8 and F-10 show that there was good retention of the TCE throughout the time course of the experiment (127 days). Figures F-9 and F-11 show DCE levels in the microcosms; these values were typically less than 400 ppb in all the bottles. The microcosms amended with yeast extract dechlorinated TCE to DCE relatively rapidly with almost complete conversion of the TCE by day 31. There were no changes in the VC or ethylene values for these microcosms. Consistent with this is the very stable value for DCE once it is formed (out to day 127). Microcosms amended with acetate and benzoate also dechlorinated but only after a considerable lag time of at least 53 days. The extent of dechlorination with acetate and benzoate was very low, with only 1.4 ppm of DCE being formed (in contrast to nearly 20 ppm with yeast extract). There was no indication of further dechlorination of the DCE as evidenced by the stable DCE values and the lack of VC formation. Samples obtained from C1-7-12 showed evidence for dechlorination in all bottles containing this sample. No dechlorination was obtained from any other sample in any other bottle tested.

4.0 CONCLUSION

Soil samples from the Paducah electro-osmosis pilot have been tested under a range of nutritional conditions for the presence of microorganisms capable of reductive dehalogenation. These conditions should generate a very broad range of anaerobic microbial types. The results show that no dechlorination was observed in any soil sample microcosm. The nutritional conditions utilized have been shown to frequently induce dechlorination in samples from other sites. Therefore, we conclude that if dechlorinating bacteria are present they could not be stimulated by these nutritional amendments.

Charcoal samples obtained from one location in the test plot exhibited dechlorination in all replicate microcosms and under both nutritional conditions tested. The results suggest perhaps a distinct microbial community within the activated charcoal traps from this specific part of the pilot. This work demonstrates that the potential is clearly there for bacteria to alter the distribution of solvents in some of the traps, but the work does not prove that this, in fact, happened *in situ*. However, it can be inferred from a comparison of solvent ratios in the different traps and the soil that the redistribution did occur *in situ*. The origin of the community is uncertain at this time given the apparent lack of activity in the soil. The bacteria may have originated with the charcoal itself and not be indigenous to the soil.

FIGURES F-2 THROUGH F-7: SOIL MICROCOSM

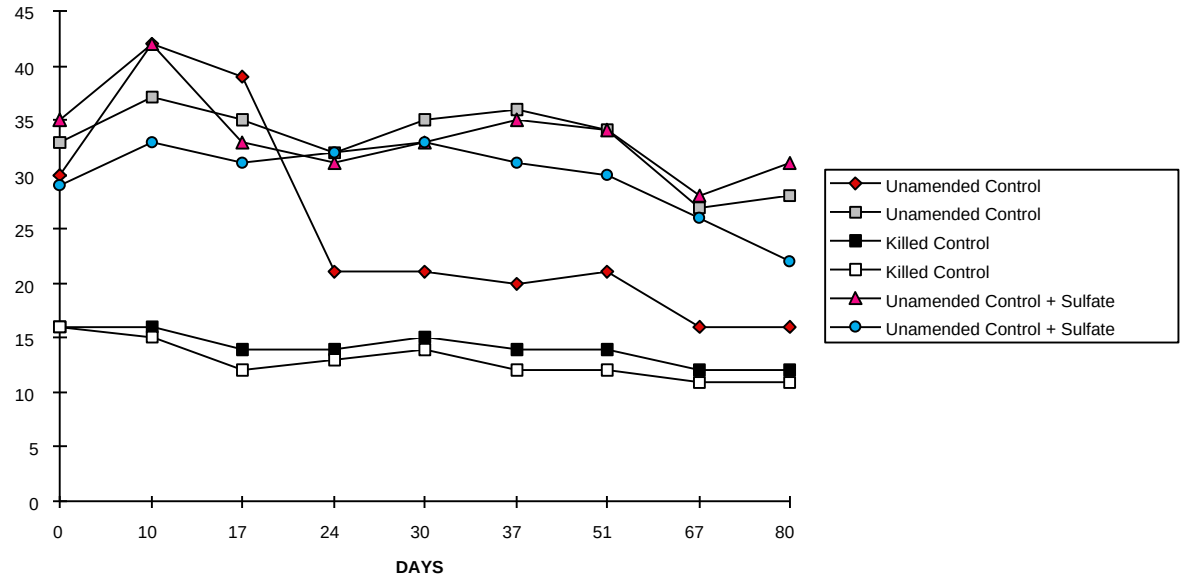

Figure F-2. Unamended and Killed Controls

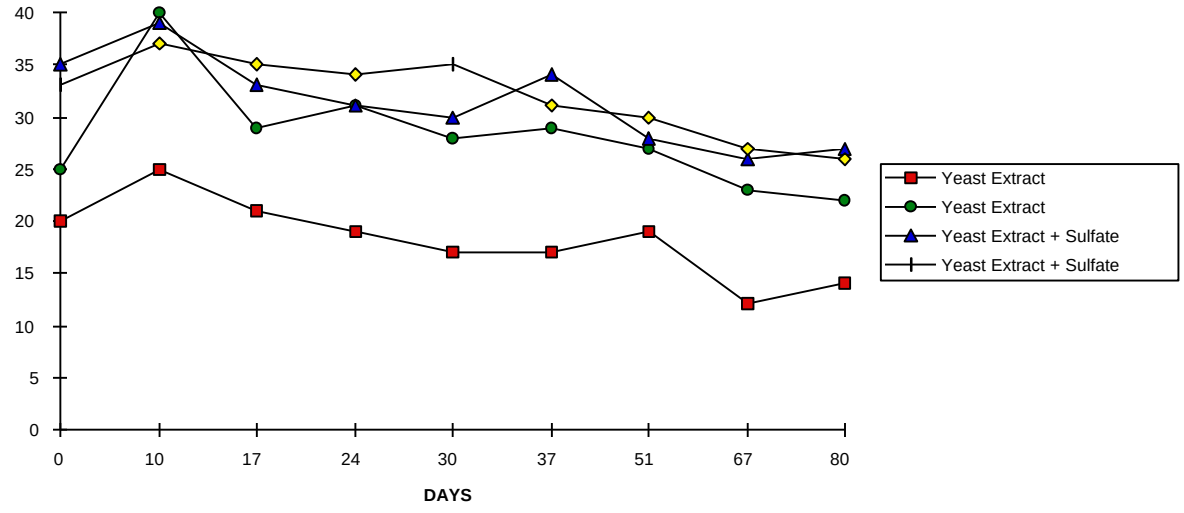

Figure F-3. Yeast Extract With/Without Sulfate

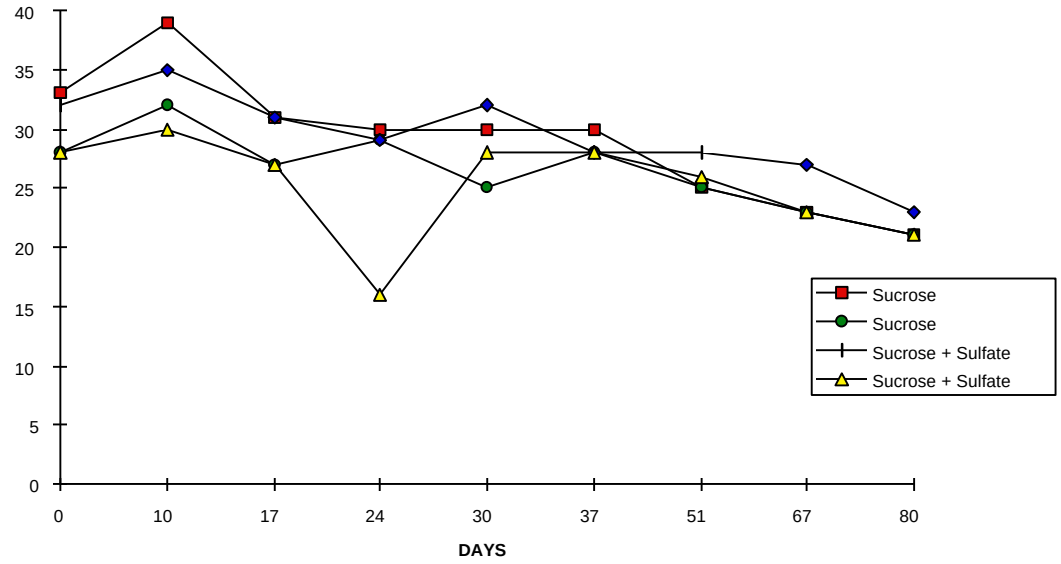

Figure F-4. Sucrose With/Without Sulfate

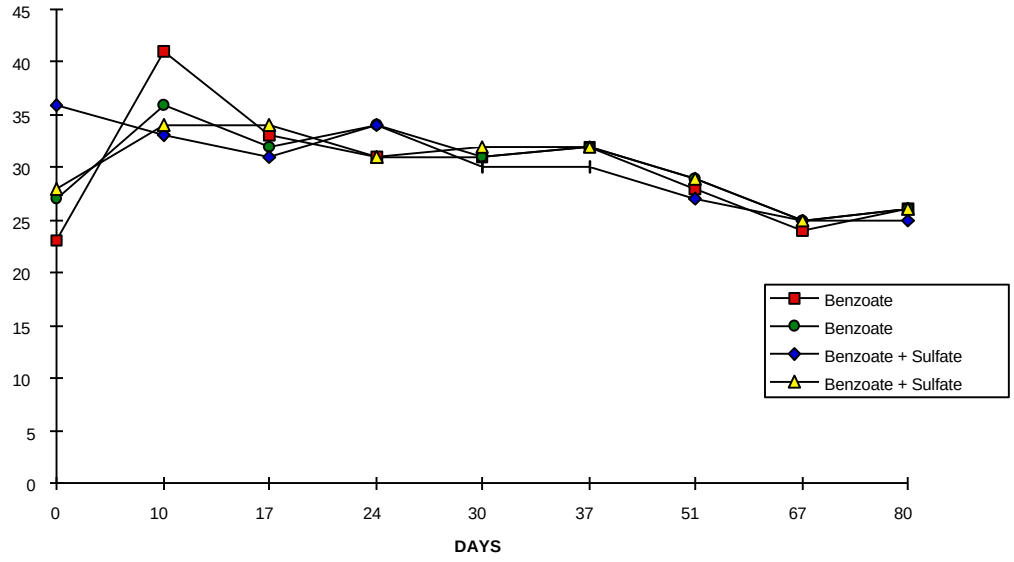

Figure F-5. Benzoate With/Without Sulfate

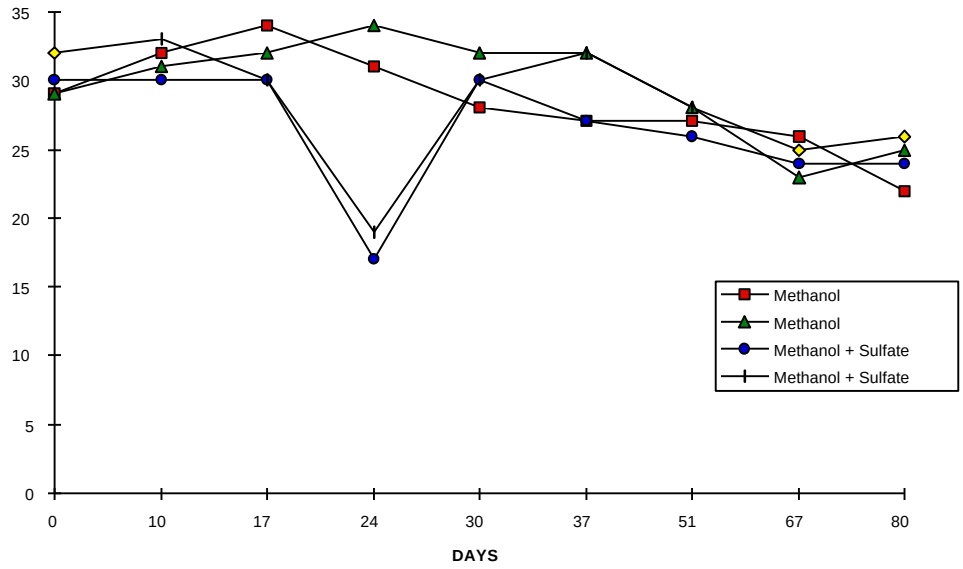

Figure F-6. Methanol With/Without Sulfate

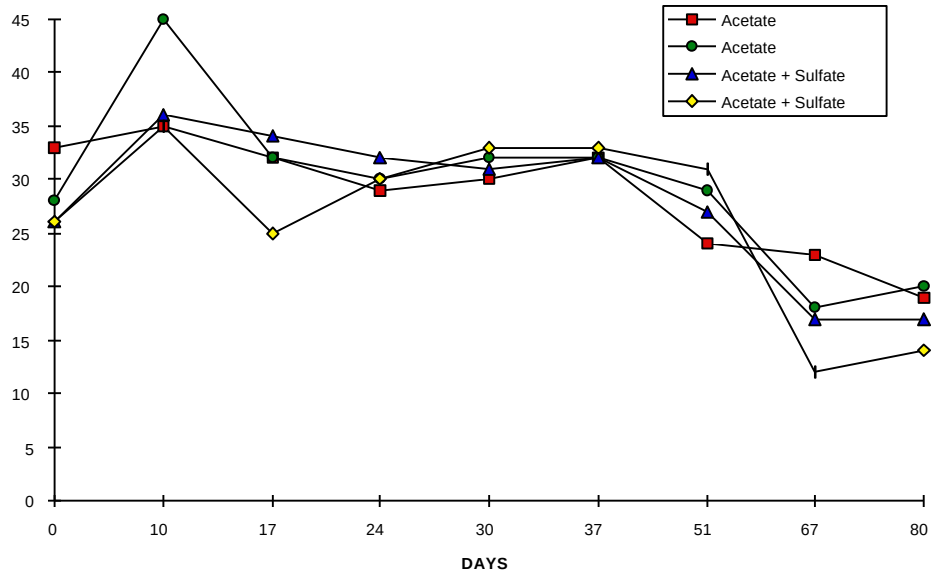

Figure F-7. Acetate With/Without Sulfate

FIGURES F-8 THROUGH F-11: CHARCOAL TRAP MICROCOSM

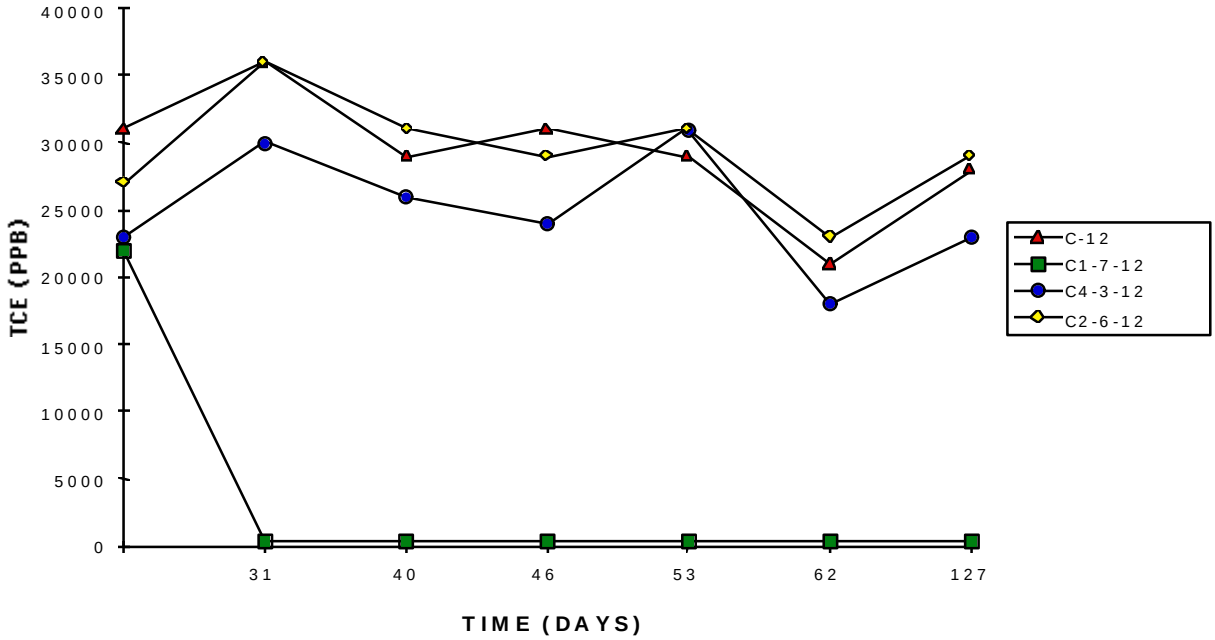

Figure F-8. TCE Levels (0.1% Yeast)

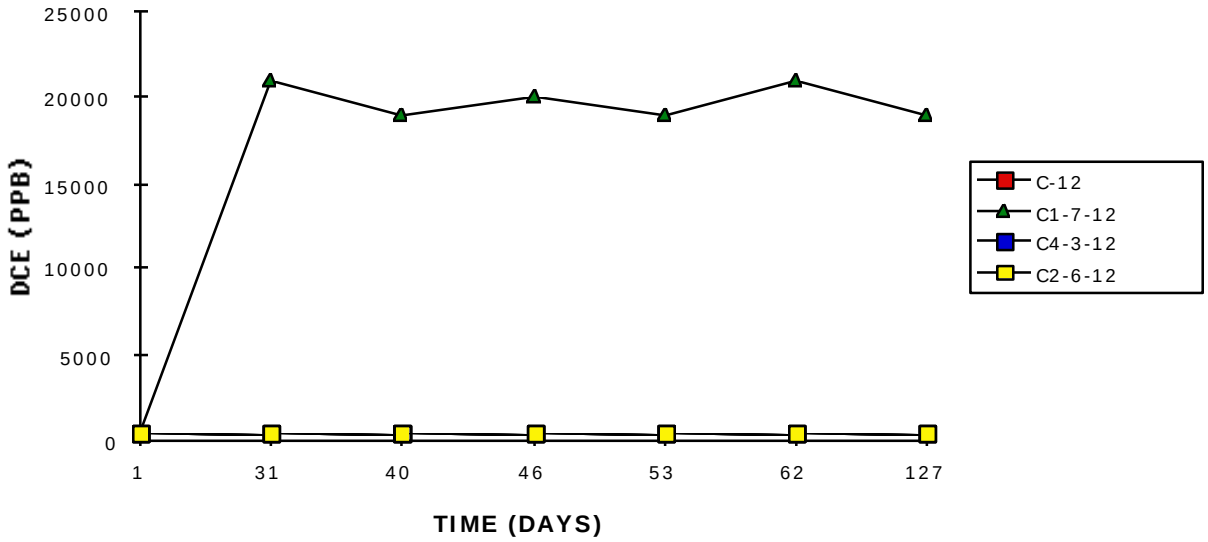

Figure F-9. DCE Levels (0.1% Yeast)

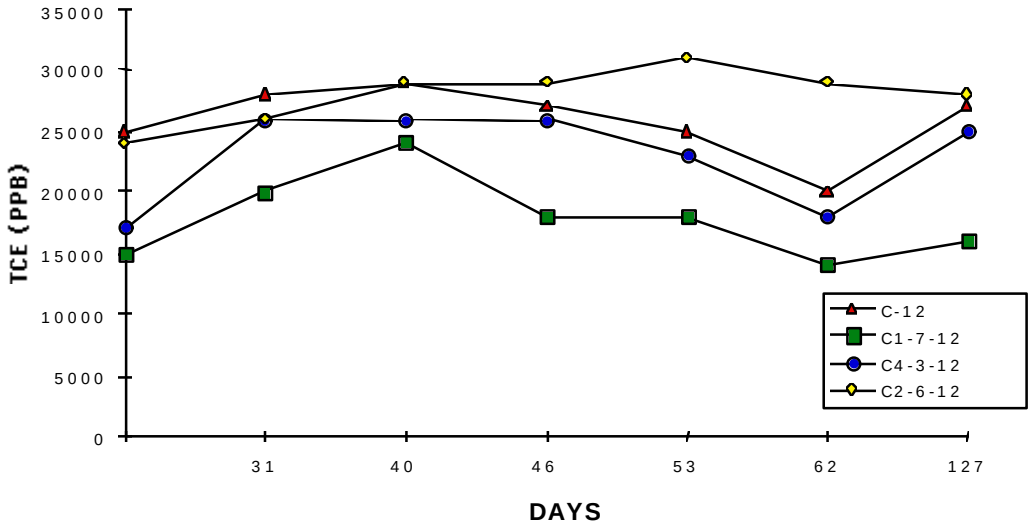

Figure F-10. TCE Levels (Benzoate and Acetate)

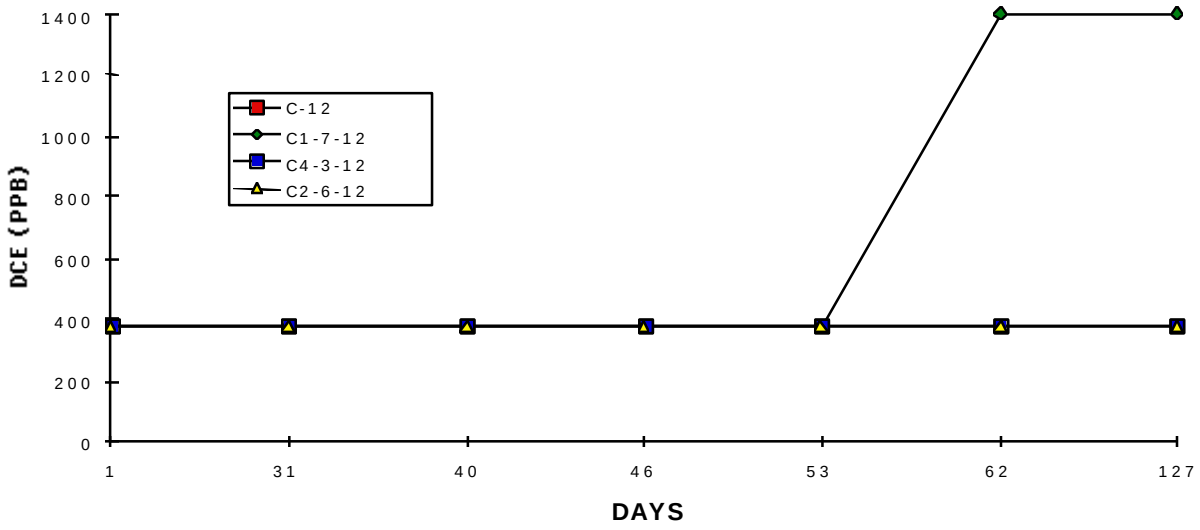

Figure F-11. DCE Levels (Benzoate and Acetate)